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# ANTIFUNGAL ACTIVITIES OF *PSIDIUM GUAJAVA* LEAVES EXTRACT AGAINST GASTROINTESTINAL FUNGAL ISOLATES

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## ABSTRACT

Current treatment of gastrointestinal problems has been faced with a lot of challenges, including antimicrobial resistance and patients' dissatisfaction, hence, the need for developing alternative cost-effective treatment approaches, one of which being screening of medicinal plants. The antifungal activities of *Psidium guajava* (guava) ethanolic leaves extracts against fungi species of gastrointestinal tract origin were studied. Fresh tender leaves of *P. guajava* were collected and extracted by ethanolic cold maceration method. Eight (8) fungal species isolated from stool specimens of gastroenteritis patients, and characterized using standard methods, were used for the study. They include: *Candida albicans, Candida parapsilosis, Candida tropicalis, Candida glabrata, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus,* and *Lentinus squarrosulus.* The antimicrobial activities of the plant leaves extract against the fungal isolates were assayed using the agar-well diffusion method, and results were analyzed statistically using statistical package for social sciences (SPSS) version 23. *Psidium guajava* leaves extract gave an inhibition zone diameter (IZD) of 6.50±0.71 mm against both *C. albicans* and *C. glabrata*, but exerted no activity (6.00±0.00) against other isolates (6.00±0.00 is equal to the diameter of the agar-well). All isolates were susceptible to clotrimazole (positive control), but resistant to the plant leaves extract, and the differences in their activities are statistically significant ($p<0.05$). Amphotericin B also exhibited significantly higher activities than the plant leaves extract ($p<0.05$). Sequel to the low antifungal activity observed in this study, it is therefore recommended that further studies be carried out on the antimicrobial (especially bacteria and fungi) and other properties of leaves extracts of *Psidium guajava* using different extraction solvents, and at varying concentrations, especially against infectious and non infectious gastroenteritis.

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**Key words**:
Antifungal activities, *Psidium guajava*, gastroenteritis

## 1.0 INTRODUCTION

Nigeria has a unique and diverse botanical heritage with over 7,895 plant species of which over 3000 species are used therapeutically (Adeleye *et.al*., 2011). While some of these plants exhibit antimicrobial effect against specific microorganisms, others show broad spectrum action against both Gram positive bacteria, Gram negative bacteria, yeasts and moulds (Okwu and Nnamdi, 2011). Over 400 medicinal plants have been known to possess antidiarrheal properties (Gupta and Birdi, 2015). The use of *Psidium guajava* (Guava) in traditional medicine for gastrointestinal problems has been reported (Gutiérrez *et al*., 2008; Chabi *et al*., 2014).

*Psidium guajava*, popularly known as guava, is a small tree belonging to the family Myrtaceae. Native to tropical areas from southern Mexico to northern South America, guava trees have been grown by many other countries having tropical and subtropical climates, thus allowing production around the world (Salazar *et.al*., 2006). Preparations of the leaves have been used in traditional medicine in several countries, mainly as anti-diarrheal remedy. Depending upon the illness, the application of the remedy is either oral or topical. The consumption of decoction, infusion, and boiled preparations is the most common way to overcome several disorders, such as diarrhea, diabetes mellitus, rheumatism, and cough, in India, China, Pakistan, and Bangladesh, while in Southeast Asia the decoction is used as gargle for mouth ulcers and as anti-bactericidal in Nigeria (Morais-Braga *et.al*., 2016; Sanda *et.al*., 2011). Aqueous and organic extracts of guava leaves have been demonstrated to have antibacterial activity due to an inhibitory effect against antibiotics-resistant clinical isolates of *Staphylococcus aureus* strains (Milyani, 2012). A methanol extract exerted antibacterial effects, preventing the growth of different strains from several bacteria such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus* spp., and *Shigella* spp. (Chah *et.al*., 2006). Furthermore, different extracts of the leaves such as aqueous, acetone–water, methanolic, spray-dried extracts, and the essential oil, showed potential inhibitory activity against Gram-positive and Gram-negative bacteria and fungi (Fernandes *et.al*., 2014).

Sequel to these numerous traditional uses of these plants, especially the common use of their tender leaves against stomach ache and diarrhea, more scientific facts are therefore needed to establish their antimicrobial activities especially against gastrointestinal fungi, so as to better understand their role in treatment of infectious gastroenteritis.

## 2.0 MATERIALS AND METHODS

### 2.1 Collection and Preparation of plant leaves

Fresh tender leaves of guava plant used for this study were collected from Ogidi metropolis in Anambra State, Nigeria, and authenticated in the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State, as *Psidium guajava*, with herbarium number, NAUH – $03^{A}$.

The leaves were washed with distilled water, and air-dried for seven days under shed, at room temperature. They were ground to powder using a hand milling machine (mechanical grinder), and the powdered samples were stored in an air-tight container. Forty grams (40 g) of the leaves powder was each extracted by cold maceration method in 400 mL of ethanol for 48 h. The extracts were filtered using muslin cloth and evaporated to dryness at 50 $^{o}$C using water bath (Selvamohan and Ramadas, 2012; Anagor *et.al*., 2019).

### 2.2 Collection and Confirmation of Isolates

Eight (8) fungal species were used for this study, which includes: *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Lentinus squarrosulus.* All isolates were gotten from stool specimens of gastroenteritis patients attending Iyi-Enu mission hospital Ogidi, Anambra state, Nigeria, and confirmed both by macroscopic, microscopic, and molecular methods.

### 2.3 Antifungal Activities Assay

Sterilized Sabouraud Dextrose agar (20 mL) was poured into sterile petri dishes, and allowed to solidify. The entire surface of the solidified culture plates were seeded each with fresh suspension of the fungal isolates adjusted to 0.5 McFarland Standard. Wells of 6 mm diameter were aseptically punched on the agar plates, and 30 uL of the crude extract (reconstituted in dimethyl sulfuroxide) was added to the wells, and allowed to stand for 30 minutes. They were incubated at 37 $^{0}$C for 24 h. Positive controls (clotrimazole and amphotericin B) and negative controls (ethanol, distilled water and DMSO) were set up similarly, and incubated. After the incubation period, zones of inhibition for duplicate analyses were measured in millimeters, and recorded as mean± standard deviation. Results were interpreted as sensitive, intermediate or resistant using the National Committee for Clinical Laboratory Standards (NCCLS) interpretative breakpoints as adopted by Magaldi *et al*. (2004).

### 2.4 Statistical Analysis

All data were analyzed using statistical package for social sciences (SPSS) version 23. Antifungal activities of the plant leaves extract against each isolate were compared using one-way Analysis of variance (ANOVA).

## 3.0 RESULTS

### 3.1 Antifungal activities of leaves extract of *P. guajava*

*Psidium guajava* leaves extract gave an inhibition zone diameter of 6.50±0.71 mm against both *C. albicans* and *C. glabrata*, but exerted no activity (6.00±0.00) against other isolates (note: 6.00±0.00 is equal to the agar well diameter). Based on NCCLS interpretive breakpoints, all isolates were resistant to the plant extract with no significant difference ($p>0.05$. see Table 1).

### 3.2 Antifungal Activity Control Results

The positive controls, clotrimazole and amphotericin B showed highest IZD of 38.00±2.83 and 18.50±2.12 against *Aspergillus fumigatus and Lentinus squarrosulus* respectively. All negative controls exerted no activities (6.00±0.00) against all the isolates (Table 2).

### 3.3 Comparison of Antifungal activities of the plant extract with clotrimazole control

All isolates were susceptible to clotrimazole but resistant to the plant extract (Table 3), and the differences are statistically significant ($p<0.05$).

### 3.4 Comparison of Antifungal activities of the plant extract with amphotericin B control

Although *Candida albicans* and *Candida parapsilosis* were resistant to amphotericin B as they also were to the plant extract, there were significant differences in the activities of amphotericin B and the plant extract against the fungal isolates (see Table 4).

Table 1. Antifungal activities of leaves extract of *P. guajava*

| Extract | *C. albicans* | *C. parapsilosis* | *C. tropicalis* | *C. glabrata* | *A. niger* | *A. flavus* | *A. fumigatus* | *L. squarrosulus* | p-value |
|---|---|---|---|---|---|---|---|---|---|
| *P. guajava* | $6.50^{R}$ ±0.71 | $6.00^{R}$ ±0.00 | $6.00^{R}$ ±0.00 | $6.50^{R}$ ±0.71 | $6.00^{R}$ ±0.00 | $6.00^{R}$ ±0.00 | $6.00^{R}$ ±0.00 | $6.00^{R}$ ±0.00 | 0.574 |

Values show mean zone of inhibition (mm) for duplicate analysis ± standard deviation. R=Resistant.

Note: NCCLS interpretive breakpoints for antifungal agents (Magaldi *et al*, 2004) are as follows:

-Susceptible (S) = ≥19 mm for azoles; ≥15 mm for non azoles

-Intermediate (I) = 18 – 13 mm for azoles; 14 – 10 mm for non azoles

-Resistant (R) = ≤12 mm for azoles; ≤9 mm for non azoles

Table 2. Antifungal Activity Control Results

| Fungal Isolates | Positive Controls | | Negative Controls | | |
|---|---|---|---|---|---|
| | Clotrimazole | Amphotericin B | Ethanol | Water | DMSO |
| *Candida albicans* | $30.00^{S}$±2.83 | $8.50^{R}$±0.71 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 |
| *Candida parapsilosis* | $30.00^{S}$±0.00 | $9.00^{R}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 |
| *Candida tropicalis* | $25.00^{S}$±2.83 | $16.00^{S}$±1.41 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 |
| *Candida glabrata* | $30.00^{S}$±1.41 | $10.00^{I}$±1.41 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 |
| *Aspergillus niger* | $29.00^{S}$±1.41 | $10.00^{I}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 |
| *Aspergillus flavus* | $30.00^{S}$±0.00 | $10.00^{I}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 |
| *Aspergillus fumigatus* | $38.00^{S}$±2.83 | $15.00^{S}$±1.41 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 |
| *Lentinus squarrosulus* | $38.00^{S}$±0.00 | $18.50^{S}$±2.12 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 | $6.00^{R}$±0.00 |

Values show mean zone of inhibition (mm) for duplicate analysis ± standard deviation

Note: NCCLS interpretive breakpoints for antifungal agents (Magaldi *et al*, 2004) are as follows:

-Susceptible (S) = ≥19 mm for azoles; ≥15 mm for non azoles

-Intermediate (I) = 18 – 13 mm for azoles; 14 – 10 mm for non azoles

-Resistant (R) = ≤12 mm for azoles; ≤9 mm for non azoles

Table 3. Comparison of Antifungal activities of the plant extract and clotrimazole control

| Fungal Isolates | Clotrimazole | *P. guajava* | p-value |
|---|---|---|---|
| *Candida albicans* | $30.00^{S}$±2.83 | $6.50^{R}$±0.71 | 0.001 |
| *C. parapsilosis* | $30.00^{S}$±0.00 | $6.00^{R}$±0.00 | - |
| *Candida tropicalis* | $25.00^{S}$±2.83 | $6.00^{R}$±0.00 | 0.002 |
| *Candida glabrata* | $30.00^{S}$±1.41 | $6.50^{R}$±0.71 | 0.001 |
| *Aspergillus niger* | $29.00^{S}$±1.41 | $6.00^{R}$±0.00 | 0.001 |
| *Aspergillus flavus* | $30.00^{S}$±0.00 | $6.00^{R}$±0.00 | - |
| *A. fumigatus* | $38.00^{S}$±2.83 | $6.00^{R}$±0.00 | 0.001 |
| *L. squarrosulus* | $38.00^{S}$±0.00 | $6.00^{R}$±0.00 | - |

Values show mean zone of inhibition (mm) for duplicate analysis ± standard deviation

Note: NCCLS interpretive breakpoints for antifungal agents (Magaldi *et al*, 2004) are as follows:

-Susceptible (S) = ≥19 mm for azoles; ≥15 mm for non azoles

-Intermediate (I) = 18 – 13 mm for azoles; 14 – 10 mm for non azoles

-Resistant (R) = ≤12 mm for azoles; ≤9 mm for non azoles

* p values are significant ($p<0.05$)

Table 4. Comparison of Antifungal activities of the plant extract and amphotericin B control

| Fungal Isolates | Amphotericin B | *P. guajava* | p-value |
|---|---|---|---|
| *Candida albicans* | $8.50^{R}$±0.71 | $6.50^{R}$±0.71 | 0.044 |
| *C. parapsilosis* | $9.00^{R}$±0.00 | $6.00^{R}$±0.00 | - |
| *Candida tropicalis* | $16.00^{S}$±1.41 | $6.00^{R}$±0.00 | 0.002 |
| *Candida glabrata* | $10.00^{I}$±1.41 | $6.50^{R}$±0.71 | 0.033 |
| *Aspergillus niger* | $10.00^{I}$±0.00 | $6.00^{R}$±0.00 | - |
| *Aspergillus flavus* | $10.00^{I}$±0.00 | $6.00^{R}$±0.00 | - |
| *A. fumigatus* | $15.00^{S}$±1.41 | $6.00^{R}$±0.00 | 0.002 |
| *L. squarrosulus* | $18.50^{S}$±2.12 | $6.00^{R}$±0.00 | 0.003 |

Values show mean zone of inhibition (mm) for duplicate analysis ± standard deviation

Note: NCCLS interpretive breakpoints for antifungal agents (Magaldi *et al*, 2004) are as follows:

-Susceptible (S) = ≥19 mm for azoles; ≥15 mm for non azoles

-Intermediate (I) = 18 – 13 mm for azoles; 14 – 10 mm for non azoles

-Resistant (R) = ≤12 mm for azoles; ≤9 mm for non azoles

* p values are significant ($p<0.05$)

## 4.0 DISCUSSION

The antifungal activities of *Psidium guajava* (guava) leaves extract against fungi species of the gastrointestinal tract were studied. Generally, *P. guajava* ethanolic leaves extract exhibited low antifungal activities against the fungal isolates used in this study. The extract gave an inhibition zone diameter of 6.50±0.71 mm against both *C. albicans* and *C. glabrata*, but exerted no activity against other isolates, especially *Aspergillus* spp. Although some authors have recorded the antibacterial and antifungal activities of medicinal plants such as *P. guajava* leaves extract (Fernandes *et al*., 2014; Tafinta *et al*., 2020), Dhiman *et al* (2011) noted that *P. guajava* leaves extract exhibited less inhibition to fungi compared to bacteria; and for *Aspergillus* spp., no activity was found (Nair and Chanda, 2007). This supports the findings of this study, as the plants extracts exhibited no activities against most of the isolates. On the other hand, Tafinta *et al* (2020) recorded higher activity of aqueous leaves extract of medicinal plants at varying concentrations against *A. niger*, than the ethanol extract. Hence, extraction method or solvent, as well as concentration of extract may affect result. This may explain the low activities observed with the crude (undiluted) ethanolic extracts used in this study.

The commercial antifungal agents (clotrimazole and amphotericin B) used as positive controls, exhibited significantly higher activities against the fungal isolates than the plant leaves extract, with clotrimazole showing the highest activities; hence, all isolates were susceptible to clotrimazole. Several antifungal agents have been in use for the treatment of fungal infections, including the azoles such as clotrimazole, and the non azoles such as amphotericin B (Benitez *et al*., 2019). Most antifungal drugs interfere with biosynthesis or integrity of ergosterol, the major sterol in the fungal cell membrane. Others cause disruption of the fungal cell wall (Chen and Sorrell, 2007). Azoles are the most widely used antifungal drugs due to their high activities, as also observed in this study. They act primarily by inhibiting the fungal cytochrome P450 enzyme, 14α-demethylase. Resistance to several antifungal agents is increasing, especially among *Candida* species and *Aspergillus* species (Pinto *et al*., 2018) as also observed in this study, with most of the isolates showing resistance to the non azole amphotericin B and the plant extract.

## 5.0 CONCLUSIONS

Crude ethanolic leaves extract of *Psidium guajava* (Guava) exhibited generally very low antimicrobial activities against selected gastrointestinal fungal isolates used in this study, especially when compared with commercially available antifungal agents.

This suggests that the common traditional use of the tender leaves of this plant in treating gastrointestinal problems may be related to an antimicrobial activity against bacteria or viral-caused gastroenteritis, or its other properties against non infectious gastroenteritis.

This study therefore recommends further studies on the antimicrobial activities and other properties of leaves extract of *Psidium guajava* using different extraction solvents, and at varying concentrations, especially against infectious and non infectious gastroenteritis.

## CONFLICT OF INTEREST

There is absolutely no conflict of interest between the authors. The study was funded by the authors.

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